

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification: A01H 5/10	A1	(11) International Publication Number: WO 00/27182 (43) International Publication Date: 18 May 2000 (18.05.2000)
(21) International Application Number: PCT/US99/26062 (22) International Filing Date: 05 November 1999 (05.11.1999) (30) Priority Data: 60/107,255 05 November 1998 (05.11.1998) US (60) Parent Application or Grant BOARD OF SUPERVISORS OF LOUISIANA STATE UNIVERSITY AND AGRICULTURAL AND MECHANICAL COLLEGE [/]; O. CROUGHAN, Timothy, P. [/]; O. CROUGHAN, Timothy, P. [/]; O. RUNNELS, John, H.; O.		Published
(54) Title: HERBICIDE RESISTANT RICE (54) Titre: RIZ RESISTANT AUX HERBICIDES (57) Abstract <p>Rice plants are disclosed with multiple sources of resistance to herbicides that normally inhibit a plant's acetohydroxyacid synthase (AHAS) enzyme. Besides controlling red rice, many AHAS-inhibiting herbicides also effectively control other weeds that are common in rice fields. Several of these herbicides have residual activity, so that one treatment can control both existing weeds and weeds that sprout later. With effective residual activity against red rice and other weeds, rice producers now have a weed control system superior to those that are currently available commercially.</p> (57) Abrégé <p>L'invention porte sur des plants de riz avec plusieurs sources de résistance aux herbicides qui, normalement, inhibent l'enzyme acétohydroxyacide synthase (AHAS) du plant. Outre leurs aptitudes de lutter contre le riz rouge, de nombreux herbicides inhibant l'AHAS luttent également de façon efficace contre d'autres mauvaises herbes courantes dans les rizières. Plusieurs de ces herbicides possèdent une activité résiduelle, de sorte qu'un traitement permet de lutter à la fois contre les mauvaises herbes existantes et contre celles qui poussent tardivement. L'activité résiduelle efficace contre le riz rouge et d'autres mauvaises herbes permet aux producteurs de disposer désormais d'un système de lutte contre les mauvaises herbes supérieur à ceux que l'on trouve actuellement dans le commerce.</p>		

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A01H 5/10	A1	(11) International Publication Number: WO 00/27182 (43) International Publication Date: 18 May 2000 (18.05.00)
(21) International Application Number: PCT/US99/26062 (22) International Filing Date: 5 November 1999 (05.11.99) (30) Priority Data: 60/107,255 5 November 1998 (05.11.98) US (71) Applicant (for all designated States except US): BOARD OF SUPERVISORS OF LOUISIANA STATE UNIVERSITY AND AGRICULTURAL AND MECHANICAL COLLEGE [US/US]; Louisiana Agricultural Experiment Station, LSU Agricultural Center, P.O. Box 25055, Baton Rouge, LA 70895-5505 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): CROUGHAN, Timothy, P. [US/US]; P.O. Box 1429, Crowley, LA 70527-1429 (US). (74) Agent: RUNNELS, John, H.; Taylor, Porter, Brooks & Phillips, L.L.P., P.O. Box 2471, Baton Rouge, LA 70821-2471 (US).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: HERBICIDE RESISTANT RICE		
(57) Abstract Rice plants are disclosed with multiple sources of resistance to herbicides that normally inhibit a plant's acetohydroxyacid synthase (AHAS) enzyme. Besides controlling red rice, many AHAS-inhibiting herbicides also effectively control other weeds that are common in rice fields. Several of these herbicides have residual activity, so that one treatment can control both existing weeds and weeds that sprout later. With effective residual activity against red rice and other weeds, rice producers now have a weed control system superior to those that are currently available commercially.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

Description

5

10

15

20

25

30

35

40

45

50

55

HERBICIDE RESISTANT RICE

5

10
15
20 The benefit of the 05 November 1998 filing date of United States provisional patent application serial number 60/107,255 is claimed under 35 U.S.C. §119(e) in the United States, and is claimed under applicable treaties and conventions outside the United States.

TECHNICAL FIELD

25 15 This invention pertains to herbicide resistant rice, particularly to rice resistant to herbicides that normally interfere with the plant enzyme acetohydroxyacid synthase (AHAS), such as imidazolinone herbicides and sulfonylurea herbicides.

BACKGROUND ART

30 20 The development of novel herbicide resistance in plants offers significant production and economic advantages. Rice production is frequently restricted by the prevalence of a weedy relative of rice that flourishes in commercial rice fields. The weed is commonly called "red rice," and belongs to the same species as cultivated rice (*Oryza sativa* L.). The genetic similarity of red rice and commercial rice has made herbicidal control of red rice difficult. The
35 25 herbicides Ordram™ (molinate: S-ethyl hexahydro-1-H-azepine-1-carbothioate) and Bolero™ (thiobencarb: S-[(4-chlorophenyl)methyl] diethylcarbamothioate) offer partial suppression of red rice, but no herbicide that actually controls red rice is currently used in commercial rice fields because of the simultaneous sensitivity of existing commercial cultivars of rice to such herbicides. The development of mutant commercial rice lines resistant to herbicides that are
40 30 effective on red rice will greatly increase the ability to control red rice infestations.

45 35 Rice producers in the southern United States typically rotate rice crops with soybeans to help control red rice infestations. While this rotation is not usually desirable economically, it is frequently necessary because no herbicide is currently available to control red rice infestations selectively in commercial rice crops. During the soybean rotation, the producer
50 has a broad range of available herbicides that may be used on red rice, so that rice may again
55

be grown the following year. United States rice producers can lose \$200 - \$300 per acre per year growing soybeans instead of rice, a potential loss affecting about 2.5 million acres annually. Additional losses in the United States estimated at \$50 million per year result from the lower price paid by mills for grain shipments contaminated with red rice. Total economic losses due to red rice in southern United States rice production are estimated to be \$500 to \$750 million a year. Economic losses due to red rice are even greater in other rice producing countries.

Rice producers typically use the herbicides propanil (trade name Stam™) or molinate (trade name Ordram™) to control weeds in rice production. Propanil has no residual activity. Molinate is toxic to fish. Neither of these herbicides controls red rice. Imazethapyr ((±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid) offers an environmentally acceptable alternative to molinate, has the residual weed control activity that propanil lacks, and is a very effective herbicide on red rice. Imazethapyr also offers excellent control of other weeds important in rice production, including barnyardgrass. Barnyardgrass is a major weed in rice production, and is currently controlled with propanil or molinate. However, there are reports that barnyardgrass is developing resistance to propanil.

The total potential market for rice varieties that are resistant to a herbicide that can control red rice is about 5.3 million acres in the United States, and the potential market outside the United States is much larger. World rice production occupies about 350 million acres. Red rice is a weed pest in rice production in the United States, Brazil, Australia, Spain, Italy, North Korea, South Korea, Philippines, Vietnam, China, Brazil, Argentina, Colombia, India, Pakistan, Bangladesh, Japan, Ecuador, Mexico, Cuba, Malaysia, Thailand, Indonesia, Sri Lanka, Venezuela, Myanmar, Nigeria, Uruguay, Peru, and in other rice-producing countries.

Herbicides that inhibit the enzyme acetohydroxyacid synthase would offer a number of advantages over currently available herbicides if they could be used in commercial rice production. Potential advantages include long residual activity against weeds, effective control of the more important weeds in rice production, including red rice, and relative environmental acceptability. Even in regions where red rice is not currently a problem, the availability of herbicide-resistant rice can have a major influence on rice production practices by providing the farmer with a new arsenal of herbicides suitable for use in rice fields.

U.S. Patent 4,761,373 describes the development of mutant herbicide-resistant maize plants through exposing tissue cultures to herbicide. The mutant maize plants were said to have an altered enzyme, namely acetohydroxyacid synthase, that conferred resistance to certain

imidazolinone and sulfonamide herbicides. See also U.S. Patents 5,304,732, 5,331,107, and 5,718,079; and European Patent Application 0 154 204 A2.

Lee *et al.*, "The Molecular Basis of Sulfonylurea Herbicide Resistance in Tobacco," *The EMBO J.*, vol. 7, no. 5, pp. 1241-1248 (1988), describe the isolation and characterization from *Nicotiana tabacum* of mutant genes specifying herbicide resistant forms of acetolactate synthase (also known as acetohydroxyacid synthase), and the reintroduction of those genes into sensitive lines of tobacco.

Saxena *et al.*, "Herbicide Resistance in *Datura innoxia*," *Plant Physiol.*, vol. 86, pp. 863-867 (1988) describe several *Datura innoxia* lines resistant to sulfonylurea herbicides, some of which were also found to be cross-resistant to imidazolinone herbicides.

Mazur *et al.*, "Isolation and Characterization of Plant Genes Coding for Acetolactate Synthase, the Target Enzyme for Two Classes of Herbicides," *Plant Physiol.* vol. 85, pp. 1110-1117 (1987), discuss investigations into the degree of homology among acetolactate synthases from different species.

U.S. Patent No. 5,767,366 discloses transformed plants with genetically engineered imidazolinone resistance, conferred through a gene cloned from a plant such as a mutated *Arabidopsis thaliana*. See also a related paper, Sathasivan *et al.*, "Nucleotide Sequence of a Mutant Acetolactate Synthase Gene from an Imidazolinone-resistant *Arabidopsis thaliana* var. Columbia," *Nucleic Acids Research* vol. 18, no. 8, p. 2188 (1990).

Examples of herbicide-resistant AHAS enzymes in plants other than rice are disclosed in U.S. Patent 5,013,659; K. Newhouse *et al.*, "Mutations in corn (*Zea mays* L.) Conferring Resistance to Imidazolinone Herbicides," *Theor. Appl. Genet.*, vol. 83, pp. 65-70 (1991); K. Sathasivan *et al.*, "Molecular Basis of Imidazolinone Herbicide Resistance in *Arabidopsis thaliana* var Columbia," *Plant Physiol.* vol. 97, pp. 1044-1050 (1991); B. Miki *et al.*, "Transformation of *Brassica napus* canola cultivars with *Arabidopsis thaliana* Acetohydroxyacid Synthase Genes and Analysis of Herbicide Resistance," *Theor. Appl. Genet.*, vol. 80, pp. 449-458 (1990); P. Wiersma *et al.*, "Isolation, Expression and Phylogenetic Inheritance of an Acetolactate Synthase Gene from *Brassica napus*," *Mol. Gen. Genet.*, vol. 219, pp. 413-420 (1989); and J. Odell *et al.*, "Comparison of Increased Expression of Wild-Type and Herbicide-Resistant Acetolactate Synthase Genes in Transgenic Plants, and Indication of Postranscriptional Limitation on Enzyme Activity," *Plant Physiol.*, vol. 94, pp. 1647-1654 (1990).

S. Sebastian *et al.*, "Soybean Mutants with Increased Tolerance for Sulfonylurea Herbicides," *Crop. Sci.*, vol. 27, pp. 948-952 (1987) discloses soybean mutants resistant to sulfonylurea herbicides. See also U.S. Patent 5,084,082.

5 K. Shimamoto *et al.*, "Fertile Transgenic Rice Plants Regenerated from Transformed
Protoplasts," *Nature*, vol. 338, pp. 274-276 (1989) discloses a genetic transformation protocol
10 in which electroporation of protoplasts was used to transform a gene encoding β -glucuronidase
into rice.

5 T. Terakawa *et al.*, "Rice Mutant Resistant to the Herbicide Bensulfuron Methyl
(BSM) by *in vitro* Selection," *Japan. J. Breed.*, vol. 42, pp. 267-275 (1992) discloses a rice
mutant resistant to a sulfonylurea herbicide, derived by selective pressure on callus tissue
15 culture. Resistance was attributed to a mutant AHAS enzyme.

Following are publications by the inventor (or the inventor and other authors)
10 concerning research on herbicide-resistant rice varieties. These publications are T. Croughan
et al., "Rice and Wheat Improvement through Biotechnology," *84th Annual Research Report*,
20 *Rice Research Station, 1992*, pp. 100-103 (1993); T. Croughan *et al.*, "Rice and Wheat
Improvement through Biotechnology," *85th Annual Research Report, Rice Research Station*,
1993, pp. 116-156 (1994); T. Croughan, "Application of Tissue Culture Techniques to the
25 Development of Herbicide Resistant Rice," *Louisiana Agriculture*, vol. 37, no. 3, pp. 25-26
(1994); T. Croughan *et al.*, "Rice Improvement through Biotechnology," *86th Annual Research
Report, Rice Research Station, 1994*, pp. 461-482 (1995); T. Croughan *et al.*, "Assessment of
Imidazolinone-Resistant Rice," *87th Annual Research Report, Rice Research Station, 1994*, pp.
30 491-525 (September 1996); T. Croughan *et al.*, "IMI-Rice Evaluations," *88th Annual Research
Report, Rice Research Station, 1996*, pp. 603-629 (September 1997); T. Croughan *et al.*,
20 "Imidazolinone-Resistant Rice," *89th Annual Research Report, Rice Research Station, 1997*, p.
464 (September 1998); T. Croughan *et al.*, "Rice and Wheat Improvement through
35 Biotechnology," USDA CRIS Report Accession No. 0150120 (for Fiscal Year 1994 – actual
publication date currently unknown); T. Croughan *et al.*, "Improvement of Lysine Content and
25 Herbicide Resistance in Rice through Biotechnology," USDA CRIS Report Accession No.
0168634 (for Fiscal Year 1997 – actual publication date currently unknown); T. Croughan,
40 "Herbicide Resistant Rice," *Proc. 25th Rice Tech. Work. Groups*, p. 44 (1994); T. Croughan *et al.*,
"Applications of Biotechnology to Rice Improvement," *Proc. 25th Rice Tech. Work.
Groups*, pp. 62-63 (1994); T. Croughan, "Production of Rice Resistant to AHAS-Inhibiting
45 Herbicides," Congress on Cell and Tissue Culture, Tissue Culture Association, *In Vitro*, vol.
30A, p. 60, Abstract P-1009 (June 4-7, 1994). (Note that the Annual Research Reports of the
Rice Research Station are published in the year after the calendar year for which activities are
50 reported. For example, the *84th Annual Research Report, Rice Research Station, 1992*,
summarizing research conducted in 1992, was published in 1993.) The reports in the *87th and*
35 *88th Annual Research Report, Rice Research Station* (published September 1996 and September

1997, respectively) mention the breeding line 93AS3510 in tables giving data on certain herbicide resistance trials. These reports gave no information on how the breeding line was developed. The breeding line was not publicly available at the times these reports were published. The breeding line 93AS3510 is the same as the ATCC 97523 rice that is described in greater detail in the present inventor's later-published international application WO 97/41218 (1997) and U.S. Patents 5,736,629, 5,773,704, and 5,952,553, and U.S. patent application serial number 09/351,889, filed July 13, 1999.

See also E. Webster *et al.*, "Weed Control Systems for Imi-Rice," p. 33 in *Program of the 27th Rice Technical Working Group Meeting* (March 1998); L. Hipple *et al.*, "AHAS Characterization of Imidazolinone Resistant Rice," pp. 45-46 in *Program of the 27th Rice Technical Working Group Meeting* (March 1998); W. Rice *et al.*, "Delayed Flood for Rice Water Weevil Control using Herbicide Resistant Germplasm," p. 61 in *Program of the 27th Rice Technical Working Group Meeting* (March 1998); E. Webster *et al.*, "Weed Control Systems for Imidazolinone-Rice," p. 215 in *Proceedings of the 27th Rice Technical Working Group Meeting* (1999); L. Hipple *et al.*, "AHAS Characterization of Imidazolinone Resistant Rice," pp. 68-69 in *Proceedings of the 27th Rice Technical Working Group Meeting* (1999); and W. Rice *et al.*, "Delayed Flood for Rice Water Weevil Control using Herbicide Resistant Germplasm," p. 134 in *Proceedings of the 27th Rice Technical Working Group Meeting* (1999).

The present inventor's U.S. Patent 5,545,822 discloses a line of rice plants having a metabolically-based resistance to herbicides that interfere with the plant enzyme acetohydroxyacid synthase; i.e., the herbicide resistance of these rice plants was not due to a resistant AHAS enzyme. (See published international application WO 97/41218, pages 6-9.) See also the present inventor's U.S. Patent 5,773,703.

The present inventor's published international application WO 97/41218 discloses one line of rice plants having a mutant AHAS enzyme that is resistant to herbicides that interfere with the wild-type plant enzyme acetohydroxyacid synthase. This line of rice plants was developed by exposing rice seeds to the mutagen methanesulfonic acid ethyl ester (EMS), and screening millions of progeny for herbicide resistance. See also the present inventor's U.S. Patents 5,736,629, 5,773,704, and 5,952,553, and U.S. patent application serial number 09/351,889, filed July 13, 1999.

U.S. Patent 4,443,971 discloses a method for preparing herbicide tolerant plants by tissue culture in the presence of herbicide. U.S. Patent 4,774,381 discloses sulfonylurea (sulfonamide) herbicide-resistant tobacco plants prepared in such a manner.

U.S. Patent 5,773,702 discloses sugar beets with a resistant mutant AHAS enzyme, derived from cell cultures grown in the presence of herbicide.

U.S. Patent 5,633,437 discloses a herbicide resistant AHAS enzyme and gene isolated from cocklebur.

U.S. Patent 5,767,361 discloses a mutant, resistant AHAS enzyme from maize. The definitions of the 5,767,361 patent are incorporated into the present disclosure by reference, to the extent that those definitions are not inconsistent with the present disclosure. See also U.S. Patent 5,731,180 and European Patent Application 0 525 384 A2.

U.S. Patent 5,605,011; European Patent Application 0 257 993 A2; and European Patent Application 0 730 030 A1 disclose resistant acetolactate synthase (ALS, another name for AHAS) enzymes based on enzymes derived from callus culture of tobacco cells in the presence of herbicide, from spontaneous mutations of the ALS gene in yeast; EMS-induced mutations in *Arabidopsis* seeds; certain modifications of those enzymes; and the transformation of various plants with genes encoding the resistant enzymes. These patents disclose several techniques for modifying AHAS genes to produce herbicide-resistant AHAS enzymes, and for transforming plants with those genes.

U.S. Patent Re 35,661 (a reissue of U.S. Patent No. 5,198,599) discloses lettuce plants with enhanced resistance to herbicides that target the enzyme acetolactate synthase. The initial source of herbicide resistance was a prickly lettuce weed infestation in a grower's field, an infestation that was not controlled with commercial sulfonylurea herbicides.

DISCLOSURE OF INVENTION

I have discovered multiple lines of novel herbicide resistance in rice plants, and improved methods for generating herbicide resistance in rice plants generally. The novel resistant rice has pre-emergence resistance, post-emergence resistance, or both pre-emergence resistance and post-emergence resistance to herbicides that are effective against red rice. The isolated novel rice lines have to date demonstrated resistance to the following herbicides: imazethapyr, imazapic, imazapyr, imazamox, sulfometuron methyl, imazaquin, chlorimuron ethyl, metsulfuron methyl, rimsulfuron, thifensulfuron methyl, pyriithiobac sodium, tribenuron methyl, and nicosulfuron. The novel rice is also expected to be resistant to derivatives of these herbicides, and to at least some of the other herbicides that normally inhibit acetohydroxyacid synthase (AHAS), particularly imidazolinone and sulfonylurea herbicides. The herbicidal activity of each of the above herbicides is known to be due to its effect on the acetohydroxyacid synthase (AHAS) enzyme. This enzyme catalyzes the first step in the synthesis of the amino acids leucine, valine, and isoleucine. Inhibition of the AHAS enzyme is normally fatal to plants.

Besides controlling red rice, many AHAS-inhibiting herbicides also effectively control other weeds commonly found in rice fields. Several of these herbicides have residual activity, so that one treatment controls both existing weeds and weeds that sprout later -- a significant advantage in rice production. No herbicide currently labelled for use on rice has residual activity against a broad spectrum of weeds including red rice. With effective residual activity against red rice and other weeds, rice producers now have a weed control system far superior to those currently used.

One role of water in rice production is in weed control -- a layer of standing water in the rice field inhibits the growth of weeds. With a herbicide having residual weed control properties, producers will have much greater flexibility in water management. Flooding of fields may now be delayed, which in turn will help control the rice water weevil, a primary insect pest of rice. Alternatively, or perhaps in conjunction, pumping costs could be reduced by delaying flooding until sufficient rain falls to flood a field at no cost to the producer.

Although the resistance mechanisms of the new rice lines have not yet been fully characterized, it is believed that the herbicide resistance of the novel rice lines is most likely attributable to different mutations of the AHAS enzyme, mutations resulting in enzymes expressing direct resistance to levels of herbicide that normally inhibit the wild-type AHAS enzyme. That the resistance is due to mutant AHAS enzymes (rather than another route such as gene copy number, enhanced promoter activity, metabolic degradation, etc.) will be confirmed using *in vitro* assays. The procedures used to assay the activity of the acetohydroxyacid synthases will be substantially as described in B.K. Singh *et al.*, "Assay of Acetohydroxyacid Synthase," *Analytical Biochemistry*, vol. 171, pp. 173-179 (1988), except as noted. In the first paragraph of Singh's "Materials and Methods," instead of corn suspension culture cells, stem tissues from greenhouse-grown rice seedlings at the four-leaf stage of development will be used. Leaf blades will be removed, and 40.0 grams (fresh weight) of tissue will be extracted in the same manner for each of the breeding lines. At the suggestion of the first author, B.K. Singh (personal communication), the desalting step mentioned at the bottom of Singh's first column under "Materials and Methods" will be eliminated. Pursuit™ herbicide (imazethapyr) will be included in the "standard reaction mixture" for the AHAS assay in various concentrations. Colorimetric absorbance will be measured at 520 nm. Checks will be made of direct acetoin formation during the enzyme assay. Each treatment will be conducted in two replicates.

An alternative AHAS assay is that disclosed in U.S. Patent 5,605,011, at col. 53, line 61 through col. 54, line 37.

MODES FOR CARRYING OUT THE INVENTION

A total of 27 new rice lines expressing resistance to AHAS-inhibiting herbicides were identified, following exposure of rice seeds to the mutagen methanesulfonic acid ethyl ester (EMS). Additional resistant rice lines will be developed and identified using similar mutation and screening techniques. Other strong mutagens known in the art may be substituted for EMS in generating such mutations, for example, nitrosoquandine, ethylnitrosourea, ionizing radiation (such as X-rays, gamma rays, or UV), or radiomimetic compounds such as bleomycins, etoposide, and teniposide. (Bleomycins, for example, are glycopeptide antibiotics isolated from strains of *Streptomyces verticillus*. One bleomycin is sold under the trademark Blenoxane[®] by Bristol Laboratories, Syracuse, NY.)

Examples 1-15

Approximately 52 million mutated (M_2) rice seed were screened. The mutated seed were developed by soaking a total of 340 pounds of seed (M_1), of the rice cultivars "Cypress" or "Bengal," in a 0.175% (by weight) aqueous solution of EMS. Approximately 170 lbs. of rice were exposed to EMS for 16 hours; approximately 85 lbs. were exposed for 24 hours; and approximately 85 lbs were exposed for 35 hours. Seed from the three exposure regimens were pooled for the screening experiments described below.

Following EMS treatment, the M_1 seed were thoroughly rinsed with water and drained before being planted by broadcast-seeding into shallow water, water that was drained 24 hours later. The field was re-flooded three days later, and the field was maintained in a flooded condition until it was drained for harvesting. The harvested M_2 seed were stored over the winter, and were screened for herbicide resistance the following spring. Following drill-seeding of the approximately 52 million M_2 seed, a pre-emergence application of imazethapyr at a rate of 0.125 lb ai/A (pounds of active ingredient per acre) was applied prior to the first flush. A post-emergence treatment of imazethapyr at 0.063 lb ai/A was applied when the rice reached the 3-leaf stage. The fifteen M_2 plants that survived the herbicide application were collected and transferred to the greenhouse.

The herbicide resistance of the progeny of these plants (M_3) was confirmed through a post-emergence application of 0.125 lb ai/A imazethapyr at the 3-leaf stage in the greenhouse. The 15 resistant plants of 52 million total M_2 plants represent a success rate of approximately 1 imidazolinone-resistant mutant identified per 3.5 million mutated seeds screened.

M_4 progeny seed were collected from the resistant M_3 plants, and were used in a field test. The field test comprised 8 replicate sets. Each of the sets contained 100 rows four feet in length. Each of the sets had 74 rows of the M_4 resistant lines. Each set had multiple rows of

each of the 15 resistant lines, with the number of rows of each of the lines varying due to the different numbers of seeds that were available for each at the time. Each of the replicate sets also contained 16 rows of the non-resistant cultivar "Cypress" as a negative control, and 10 rows of earlier-developed herbicide-resistant rice lines as positive controls. (The positive controls were either ATCC 97523 or a hybrid of ATCC 97523 and ATCC 75295.)

A different herbicide treatment was applied post-emergence to each of these eight replicate sets when the rice reached the 3 leaf stage. The control set was treated with 4 quarts/acre of Arrosolo™. Arrosolo™ is a herbicide that is currently used commercially with conventional rice varieties. The remaining 7 sets were treated with imidazolinone herbicides as follows: (1) imazethapyr (trade name Pursuit™) at 0.125 lb ai/A; (2) imazethapyr at 0.188 lb ai/A; (3) imazapic (trade name Cadre™) at 0.063 lb ai/A; (4) imazapic at 0.125 lb ai/A; (5) imazapyr (trade name Arsenal™) at 0.05 lb ai/A; (6) imazapyr at 0.09 lb ai/A; and (7) a mixture of 75% imazethapyr and 25% imazapyr (trade name Lightning™) at 0.052 lb ai/A.

Note that all herbicide application rates tested were equal to or greater than the recommended application rates for the use of the same herbicides on other crops.

Levels of resistance to herbicide were determined both at three weeks after spraying, and at maturity. No row was significantly injured by the control treatment with the conventional rice herbicide Arrosolo™. By contrast, each of the seven imidazolinone treatments resulted in 100% control of the rows of non-resistant Cypress rice, without a single surviving plant among any of the 112 treated rows. Each of the herbicide-resistant M₄ progeny rows in each of the sets, and each of the herbicide-resistant positive controls in each of the sets, displayed insignificant injury or no injury from the various imidazolinone treatments. The rows of resistant M₄ progeny treated with the imidazolinones, and the rows of herbicide-resistant positive controls treated with the imidazolinones, were visually indistinguishable from the Arrosolo™-treated rows with respect to height, vigor, days to maturity, and lack of visible herbicide injury.

Samples of the seed harvested from each of the fifteen lines of the M₄ progeny, i.e., samples of M₄ seed from each of the fifteen separate lines; lines designated by the inventor as SSC01, SSC02, SSC03, SSC04, SSC05, SSC06, SSC07, SSC08, SSC09, SSC10, SSC11, SSC12, SSC13, SSC14, and SSC15; were separately deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia 20110-2209 on November 5, 1998; and were assigned ATCC Accession Nos. 203419, 203420, 203421, 203422, 203423, 203424, 203425, 203426, 203427, 203428, 203429, 203430, 203431, 203432, and 203433, respectively. Each of these deposits was made pursuant to a contract

5 between ATCC and the assignee of this patent application, Board of Supervisors of Louisiana
State University and Agricultural and Mechanical College. Each of the contracts with ATCC
10 provides for permanent and unrestricted availability of these seeds or the progeny of these
5 seeds to the public on the issuance of the U.S. patent describing and identifying the deposit or
the publication or the laying open to the public of any U.S. or foreign patent application,
15 whichever comes first, and for the availability of these seeds to one determined by the U.S.
Commissioner of Patents and Trademarks (or by any counterpart to the Commissioner in any
patent office in any other country) to be entitled thereto under pertinent statutes and
20 regulations. The assignee of the present application has agreed that if any of the seeds on
deposit should become nonviable or be lost or destroyed when cultivated under suitable
conditions, they will be promptly replaced on notification with a viable sample of the same
seeds.

Examples 16-27

25 15 Approximately 60 million additional mutated (M_2) rice seed were screened. The
mutated seed were developed by soaking a total of 300 pounds of seed (M_1) of the rice cultivar
"Cypress" in a 0.175% (by weight) aqueous solution of the mutagen EMS for 23 hours.

30 Following EMS treatment the M_1 seed were thoroughly rinsed with water and drained
before being planted by broadcast-seeding into shallow water, water that was drained 24 hours
20 later. The field was re-flooded three days later, and the field was maintained in a flooded
condition until it was drained for harvesting. The harvested M_2 seed were stored over the
winter, and were screened for herbicide resistance the following spring. Following broadcast-
35 seeding and shallow soil incorporation of approximately 60 million M_2 seed, a post-emergence
application of imazapic (trade name Cadre™) at 0.125 lb ai/A was sprayed on half the field,
25 and a post-emergence application of imazapyr (trade name Arsenal™) at 0.10 lb ai/A was
applied to the remaining half of the field at the three-leaf stage. The twenty-three M_2 plants that
40 survived the herbicide application were collected and transferred to the greenhouse. Later
testing (described below) showed that twelve of these plants represented new herbicide resistant
lines; the other plants were either "escapes" (plants receiving no herbicide spray), or
45 30 "volunteer" seed of the ATCC 97523 line that had remained in the soil from a prior season.

The 12 resistant plants of 60 million total M_2 plants represent a success rate of
approximately 1 imidazolinone-resistant mutant identified per 5 million mutated seeds screened.

50 The herbicide resistance of the progeny of these plants (M_3) was confirmed with the
following herbicide applications in the greenhouse: 0.125 lb ai/A imazethapyr (trade name
35 Pursuit™) as a pre-emergence application; 0.063 lb ai/A imazethapyr as a post-emergence

5 application; 0.10 lb ai/A sulfometuron methyl (trade name Oust™) as a pre-emergence application; 0.05 lb ai/A sulfometuron methyl as a post-emergence application; 0.10 lb ai/A
10 nicosulfuron (trade name Accent™) applied pre-emergence; and 0.05 lb ai/A nicosulfuron applied post-emergence. Two M₃ seed from each of the twenty-three herbicide-resistant lines
5 were planted in each of four replicate pots for each treatment. Equivalent plantings of control lines were made with (non-resistant) Cypress and Bengal rice seeds.

15 Samples of the seed harvested from several of these lines of the M₄ progeny; namely, samples of M₃ seed from each of the seven separate lines designated by the inventor as PWC16, PWC23, CMC29, CMC31, WDC33, WDC37, and WDC38; were separately
10 deposited with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia 20110-2209 on November 2, 1999; and were assigned ATCC Accession Nos. aaaaaa, bbbbbb, cccccc, dddddd, eeeee, fffff, and ggggg, respectively. Each of these deposits
20 was made pursuant to a contract between ATCC and the assignee of this patent application, Board of Supervisors of Louisiana State University and Agricultural and Mechanical College.
25 Each of the contracts with ATCC provides for permanent and unrestricted availability of these seeds or the progeny of these seeds to the public on the issuance of the U.S. patent describing and identifying the deposit or the publication or the laying open to the public of any U.S. or
30 foreign patent application, whichever comes first, and for the availability of these seeds to one determined by the U.S. Commissioner of Patents and Trademarks (or by any counterpart to the
20 Commissioner in any patent office in any other country) to be entitled thereto under pertinent statutes and regulations. The assignee of the present application has agreed that if any of the
35 seeds on deposit should become nonviable or be lost or destroyed when cultivated under suitable conditions, they will be promptly replaced on notification with a viable sample of the same seeds.

25 Five other lines, designated by the inventor as PWC17, PWC19, PWC21, PWC22, and CMC27, exhibited lower levels of herbicide resistance. These lines appear to differ both from
40 the lines that have now been deposited with ATCC, and from prior line ATCC 97523. Due to their lower levels of resistance, these lines had not been deposited with ATCC as of the international filing date of the present application. However, these lines may have potential
45 30 value as breeding material to cross with other sources of herbicide resistance, or with each other, in order to enhance total levels of resistance. If these five lines involve different resistance mechanisms, or different AHAS isozymes as compared to the ATCC-deposited lines,
50 then crossing one of these lines with one of the ATCC-deposited lines could result in a hybrid with an enhanced total level of resistance. Their herbicide resistance levels would not,

however, appear to make any of these five lines, standing alone, suitable candidates for breeding new herbicide resistant rice lines.

Further Field Tests and Greenhouse Tests

Further field tests and greenhouse tests were conducted to evaluate the tolerance of the resistant lines. The field tests included both pre-emergence and post-emergence herbicide application studies. The same lines were included in both studies, except that line WDC37 was included in the pre-emergence study only, due to the lack of sufficient quantity of seed at the time.

The herbicides applied as pre-emergence applications were imazaquin, imazethapyr, and imazapic. Each treatment was applied to each of two replicate plots. Each replicate plot contained three-foot long rows of each herbicide resistant line, along with a check row of non-resistant rice. Two plots were left unsprayed to serve as untreated controls. All herbicide-resistant lines exhibited little or no injury from the herbicide applications. All check rows of the non-resistant rice variety Cypress, by contrast, were either killed or severely injured in all plots given herbicide treatments.

Post-emergence application was studied in fifty replicate plots of the same herbicide-resistant lines, except that line WDC37 was not included in the post-emergence field study. For the post-emergence field study, each herbicide treatment was applied to each of two replicate plots. Four plots were left unsprayed to serve as untreated controls. Herbicide treatments studied post-emergence were imazethapyr (Pursuit™), imazapic (Cadre™), imazamox (Raptor™), a 1:1 (by weight) mixture of imazapic and imazapyr, a 3:1 (by weight) mixture of imazapic (Cadre™) and imazapyr (Arsenal™), imazapyr (Arsenal™), chlorimuron ethyl (Classic™), metsulfuron methyl (Ally™), nicosulfuron (Accent™), rimsulfuron (Matrix™), a 2:1 mixture (by weight) (Harmony Extra™) of thifensulfuron methyl and tribenuron methyl, and pyriithiobac sodium (Staple™).

The greenhouse tests comprised two replicate studies using the same herbicides and rates as were used in the post-emergence field test. The greenhouse studies evaluated the post-emergence herbicide resistance of a few lines for which the quantity of seed then available was inadequate to include in the field tests. Seeds of the resistant lines were planted in 2 inch x 2 inch peat pots, and the seedlings were then sprayed at the 3-4 leaf stage. Non-resistant check

lines were included for comparison. As in the field tests, the non-resistant checks were either killed or severely injured by the herbicide treatments.

The results of these field and greenhouse studies are summarized in Tables 3 and 4.

RESULTS AND DISCUSSION

Previous selections for imidazolinone-resistant rice by screening following seed exposure to EMS had resulted in fewer resistant rice lines. For example, screening approximately 35 million M_2 seed following exposure of the M_1 seed to 0.5% EMS for 16 hours resulted in a single herbicide-resistant mutant plant, for a success ratio of 1 resistant mutant per 35 million mutated seed. By contrast, each of the two series of screenings reported here had a significantly higher rate of successfully producing herbicide-resistant mutants. It is believed, without wishing to be bound by this theory, that the improved efficiency was due to the difference in mutagen concentrations and exposure times used.

The more efficient mutation protocols described here used a relatively longer exposure to a relatively lower concentration of mutagen than had previously been used. In Examples 1-15 the average mutagen exposure time was 22.75 hours, and the EMS concentration was 0.175%. This represents a 42% longer average exposure time, and a 65% reduction in the mutagen concentration, as compared to the only successful event from the earlier screening of 35 million seeds. The result was a ten-fold increase in the rate of resistant mutant recovery (one per 3.5 million seed versus one per 35 million seed).

Examples 16-27 used conditions similar to those for Examples 1-15, and were also more efficient in producing resistant mutants. The same EMS mutagen concentration (0.175%) was used, and only a slightly different exposure time (23 hours versus an average of 22.75 hours). The herbicide-resistant mutant production rate in this trial was 1 plant per 5 million seed. These results indicate that longer exposures to lower mutagen concentrations appear generally to produce higher rates of successful herbicide resistant mutants.

Each of the resistant mutants from these two screenings exhibits resistance to one or more imidazolinone and sulfonylurea herbicides. A summary of the herbicide application used in the initial screening for resistance is given in Table 1. The results of the field tests for Examples 1-15 (SSC01 through SSC15) are given in Table 2. The results of the field tests for Examples 16-27 (those resistant lines having PWC, CMC, or WDC designations) are given in Tables 3 and 4. Note that the application rates in Tables 1, 2, and 3 are given in pounds of

active ingredient per acre, while the rates in Table 4 are given in ounces of active ingredient per acre.

Table 1 – Screening Herbicide Application

Line	Screening Herbicide Application (lb ai/A)		
	Imazethapyr 0.125 pre-emerge + 0.063 post-emerge	Imazapyr 0.10 post-emerge	Imazameth 0.125 post-emerge
SSC01	X		
SSC02	X		
SSC03	X		
SSC04	X		
SSC05	X		
SSC06	X		
SSC07	X		
SSC08	X		
SSC09	X		
SSC10	X		
SSC11	X		
SSC12	X		
SSC13	X		
SSC14	X		
SSC15	X		
PWC16		X	
PWC17		X	
PWC18		X	
PWC19		X	
PWC20		X	
PWC21		X	
PWC22		X	

5		PWC23		X	
		PWC24		X	
10		CMC25			X
		CMC26			X
5		CMC27			X
		CMC28			X
15		CMC29			X
		CMC30			X
		CMC31			X
20	10	WDC32			X
		WDC33			X
		WDC34			X
		WDC35			X
25		WDC36			X
	15	WDC37			X
		WDC38			X

30

35

40

45

50

55

Table 2 -- Post-Screening Herbicide Testing

Herbicide Application Rate (lb ai/A); & whether applied pre-emergence or post-emergence														
Line	Imazethapyr				Imazapyr		Imazameth		Imazethapyr (75%) + Imazapyr (25%)		Sulfometuron Methyl		Nicosulfuron	
	0.125 pre	0.063 post	0.125 post	0.188 post	0.05 post	0.09 post	0.063 post	0.125 post	0.052 post	0.10 pre	0.05 post	0.10 pre	0.05 post	
SSC01	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC02	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC03	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC04	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC05	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC06	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC07	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC08	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC09	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC10	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC11	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC12	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC13	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC14	X	X	X	X	X	X	X	X	X	X	0	0	X	X
SSC15	X	X	X	X	X	X	X	X	X	X	0	0	X	X

PWC16	X	X	0	0	X	X	X
PWC17	X	X	X	0	X	X	X
PWC18	X	X	0	0	X	X	X
PWC19	X	X	0	0	X	X	X
PWC20	X	X	0	0	X	X	X
PWC21	X	X	0	0	X	X	X
PWC22		X					
PWC23	X	X	X	X	X	X	X
PWC24	X	X	X	0	X	X	X
CMC25	X	X	X	0	X	X	X
CMC26	X	X	0	0	X	X	X
CMC27							
CMC28	X	X	0	0	X	X	X
CMC29	X	X	0	0	X	X	X
CMC30	X	X	0	0	X	X	X
CMC31	X	X	0	0	X	X	X
WDC32	X	X	X	X	X	X	X
WDC33	X	X	0	0	X	X	X
WDC34	X	X	X	0	X	X	X
WDC35	X	X	0	0	X	X	X
WDC36	X	X	0	0	X	X	X
WDC37	X	X	0	0	X	X	X

18

55[illegible]

Notes to Table 2: X = sensitive (exhibited wild-type reaction to herbicide); blank = not yet tested.

Herbicide Application Rate (lb ai/A); & whether applied pre-emergence or post-emergence																			
Imazethapyr						Imazapic						Imazaquin				Imamazox		Imazapic 0.05 + Imazapyr 0.05	
Line	0.063 pre	0.125 pre	0.188 pre	0.063 post	0.125 post	0.037 pre	0.075 pre	0.15 pre	0.075 post	0.15 post	0.125 pre	0.25 pre	0.375 pre	0.05 post	0.10 post				
SSC01	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
SSC02	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
SSC03	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
SSC04	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
SSC05	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
SSC06	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
SSC07	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	X			X
SSC08	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
SSC09	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
SSC10	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
SSC11	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
SSC12	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X
SSC13	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	X			X
SSC14	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	X			X

[illegible]

Notes to Table 3: X = sensitive (exhibited wild-type reaction to herbicide); blank = not yet tested.

Table 3 (cont.) - Post-Screening Herbicide Testing

Line	Herbicide Application Rate (lb ai/A); & whether applied pre-emergence or post-emergence			
	Imazapic 0.075 + Imazapyr 0.025		Imazapic 0.15 + Imazapyr 0.05	
	post		post	
SSC01	X		X	X
SSC02	X		X	X
SSC03	X		X	X
SSC04	X		X	X
SSC05	X		X	X
SSC06	X		X	X
SSC07	X		X	X
SSC08	X		X	X
SSC09	X		X	X
SSC10	X		X	X
SSC11	X		X	X
SSC12	X		X	X
SSC13	X		X	X
SSC14	X		X	X
SSC15	X		X	X

PWC16	X	X	X	X	X
PWC23	X	X	X	X	X
CMC29	X	X	X	X	X
CMC31	X	X	X	X	X
WDC33	X	X	X	X	X
WDC37					
WDC38	X	X	X	X	X

Notes to Table 3: X = resistant; 0 = sensitive (exhibited wild-type reaction to herbicide); blank = not yet tested.

Table 4 – Post-Screening Herbicide Testing

[illegible]

SSC14	X	X	X	X	X	X	X	X	X	X	X	X
SSC15	X	X	X	X	X	X	X	X	X	X	X	X
PWC16	0	0	X	X	0	0	0	X	X	X	X	X
PWC23	0	0	X	X	0	0	0	X	X	X	X	X
CMC29	0	0	0	X	0	0	0	X	X	X	X	X
CMC31	0	0	0	X	X	0	0	X	X	X	X	X
WDC33	X	X	X	X	0	0	0	X	X	X	X	X
WDC37												
WDC38	0	0	0	X	X	0	0	0	X	X	X	X

Notes to Table 4: X = sensitive (exhibited wild-type reaction to herbicide); blank = not yet tested. In the entries for CMC29, CMC31, and WDC38 for mesulfuron methyl, and also for WDC38 (only) for the thifensulfuron methyl - tribenuron methyl mixture, at the lower rate of application, the response was identical to that of the wild-type, with all surviving the lower rate of application; while at the higher rate of application, the wild-type plants were seriously injured, and the CMC29, CMC31, and WDC38 lines exhibited substantially less injury.

Further examination of these plants led to the conclusion that the following herbicide resistant lines appeared to be identical to prior herbicide resistant line ATCC 97523, presumably because a few seeds of ATCC 97523 from prior trials had remained dormant in the soil between growing seasons: PWC18, PWC20, PWC24, CMC25, CMC26, CMC28, CMC30, WDC32, WDC34, WDC35, and WDC36.

Enhanced resistance will result from crossing the novel rice lines with one another. Enhanced resistance will also result from the synergy of crossing one or more of the novel rice lines, with their resistant AHAS enzymes, with the metabolic-based resistant rice lines disclosed in U.S. Patent 5,545,822, as typified by the rice having ATCC accession number 75295. As disclosed in the present inventor's published international application WO 97/41218, such synergy has been seen in hybrids of the rice having ATCC accession number 75295 with the rice having ATCC accession number 97523, the latter having a mutant, resistant AHAS enzyme in rice.

Notes on Mutation Selection Procedures in the Field

The following procedures were used for screening large quantities of mutated rice seed for herbicide resistance in the field.

Exposure to mutagen or to conditions conducive to the induction of mutations may be performed at different stages of growth and different culture conditions, e.g., exposing dry seed, seed sprouted in water for 24 hours, or seed sprouted in water for 48 hours, etc. to mutagen; or growing cells in tissue culture, such as anther culture, with or without the application of mutagen; etc.

Rice to be planted for seed is ordinarily cleaned after harvest. Once cleaning is completed, any standard planting equipment can be satisfactorily used. However, this laborious and time-consuming cleaning step can be bypassed if the planting equipment will tolerate the pieces of straw and other extraneous material that typically accompany combine-harvested rice. Eliminating the cleaning step allows generations of seed to be grown, screened, and increased more rapidly. For example, using a spinner/spreader attachment on a tractor allows broadcast planting of rice that is accompanied by a moderate amount of extraneous material. Broadcast planting is also more rapid than drill-seeding, saving further time and labor. Seed planted with

5 a spinner/spreader can either be lightly incorporated into the soil following broadcast-spreading, or allowed to remain on the soil surface, in which case it must be kept sufficiently moist by irrigation if rainfall is inadequate.

10
5 Freshly-harvested rice seed may have a degree of dormancy, which prevents some of the otherwise viable seed from sprouting immediately. This dormancy normally disappears during storage. However, if the harvested seed is to be planted for selection purposes shortly after harvest to accelerate generation time, then treatment to reduce or eliminate dormancy is beneficial. One method to eliminate dormancy is to expose the seed to a temperature of about 15 50°C for about five days. Moisture should be allowed to escape from the seed during this treatment, so relatively small containers of moisture-permeable material should be used, such as cloth bags. Alternatively, stems with panicles still attached may be positioned to allow air to circulate over the panicles, for example, by standing them upright in a paper bag. As a further alternative, forced-air drying may be used, with or without storage in bags, provided that the seed is situated so that moisture is not entrapped around sections of the grain.

20 When spraying mutated rice seed or plants to identify resistant individuals, it is important to achieve as uniform and precise a treatment as possible. Since the number of true resistant individuals will be a very small fraction of the total number of seeds, even a small fraction of "escapes" (i.e., false positives, plants fortuitously not receiving any herbicide) can complicate and retard the screening process. Therefore the herbicide-spraying equipment should be in good condition, and should be calibrated as accurately as possible. Each spray nozzle along the spraying boom should deliver spray at the same volumetric rate. Nozzles should be accurately aligned to avoid insufficient spray overlap between nozzles. Relatively short tractor spray booms (for example, approximately 12 feet) are helpful in minimizing undesirable boom movements while spraying.

40
45 30 Appropriate nozzles include the following, each of which has a flat spray tip, and sprays approximately 15 gallons per acre at 40 pounds per square inch (gauge) spray pressure, with a 20-inch nozzle spacing, at the indicated ground speeds: 8001VS (2 mph), 80015VS (3 mph), 8002VS (4 mph), 8003VS (5 mph). (Spraying Systems Co., Wheaton, Illinois) To optimize the spray pattern, the nozzle height above the target (either the top of the plant canopy or the soil) should be adjusted so that the spray pattern from each nozzle overlaps the spray pattern from each adjacent spray nozzle by about 30% (as measured linearly). Using the 80 degree nozzles listed above, at a 40 psi spraying pressure, and a 20 inch spacing between

nozzles, the correct spray height above the target would be 17 to 19 inches. Holding other parameters constant, but changing the nozzle spacing to 30 inches, the correct spray height would increase to 26 to 28 inches. Using spray pressures lower than 40 psi will typically reduce the nozzle spray angles, and adjusting to a lower spray height may be necessary to achieve proper overlap at lower pressures. All spray equipment should be precisely calibrated before use.

When spraying, carefully measured marking flags to guide the spray-rig operator are frequently beneficial, as are flags at midfield in larger fields, in addition to those at the ends of the fields. Wind speed should be essentially zero, a condition that is often seen in the early morning or late afternoon. Spraying should not be performed if rain is anticipated within about the next six hours (a time that varies, depending on the particular herbicide). Pre-emergence spraying should be applied to dry ground. If the herbicide requires moisture for activation, then irrigation or rainfall after planting is required.

Uniformity of spraying is best accomplished by dividing the herbicide to be applied equally between two consecutive sprayings, one after the other. The spray solution is prepared at half the final treatment concentration. Two passes are then made in opposite directions to achieve the desired total treatment concentration. For example, if the first pass on a particular row is made in the North-to-South direction, the second pass is made in the South-to-North direction. When spraying with a tractor, this may be accomplished by traveling in the opposite direction in the same tracks for the second application.

Complete coverage is promoted by using large spray volumes (i.e., dilute concentrations of herbicide) and small spray droplet size. Spray volumes of 30 to 40 gallons per acre have worked well, particularly with two applications of 15 to 20 gallons per acre each. Spray pressures of 30 to 40 pounds per square inch (gauge) have worked well in producing fine sprays that provide thorough coverage. Nozzles should be evenly spaced, preferably about 20 inches apart.

The total rate of herbicide application used for the selection is preferably at least twice the normal use rate for that herbicide. For example, if 0.063 lb ai/A is the normal use rate for crops, then an appropriate concentration to select for resistant individuals would be two applications at the same rate, resulting in 0.125 lbs ai/A total treatment.

5 The combination of two sprayings, large spray volumes, high spray pressures, and an
elevated treatment concentration helps minimize the occurrence of escapes, i.e., individuals that
10 are not truly resistant, but that survived the procedure simply because they were inadequately
sprayed.

5 There are advantages to conducting selection with herbicides that possess both soil and
foliar activities. The soil activity of the herbicide can be used directly to select for resistant
15 individuals that grow despite the pre-emergence application. Alternatively, a pre-emergence
application can be used to eliminate a large percentage of the non-resistant entities, following
10 which a foliar application is made to the surviving individuals. This early thinning of the stand
density greatly reduces the problem of spray interception that can otherwise occur within a
20 thick stand of young seedlings, i.e., the possibility of a seedling that is physically shielded
from the spray by other seedlings.

25 15 Using both soil and foliar application of a suitable herbicide also reduces the problem
of "escapes," because the herbicide's soil activity will often eliminate individuals that escape
the foliar spray. When using a herbicide having primarily, or only, foliar activity, an
additional spraying may be necessary for two reasons. One reason is to eliminate non-resistant
30 individuals that escaped the foliar spray. Also important is the elimination of non-resistant
20 individuals originating from late-sprouting seed. A plant that grows from a seed that sprouts
after spraying will not be controlled by a herbicide having only foliar activity. Within two
weeks, such a plant may reach a size that makes it appear to be a resistant mutant that survived
35 the foliar treatment. If a second foliar spraying is either undesirable or not possible, an
alternative is to leave a small area of the field unsprayed when applying the first application, to
25 provide a direct standard for determining the size that resistant seedlings should achieve during
the intervening period.

40 Using a herbicide with both soil and foliar activity also presents the opportunity to
select efficiently for both pre-emergence and post-emergence resistance within the same
45 30 individual. This selection is accomplished by applying sequential applications. The likelihood
is high that individuals surviving sequential applications are resistant to both pre and post-
emergence treatments with that herbicide.

50 As the selection procedure is in progress, care should be taken that the few surviving
35 individuals are not eaten by birds or insects. This is particularly important when using post-

emergence treatments, but is also important with pre-emergence treatments. Sound-making devices may be used to drive away birds such as blackbirds, which consume rice seeds and small seedlings. Insects such as fall armyworms and rice water weevils also may kill small survivors, and the application of an insecticide on a preventative basis is frequently desirable. Daily monitoring of the situation should be undertaken if an investigator chooses not to use bird-discouraging devices or insecticides preventatively.

Miscellaneous

Through routine breeding practices known in the art, progeny will be bred from each of the resistant parent lines identified above. Once progeny are identified that are demonstrably resistant, those progeny will be used to breed varieties for commercial use. Crossing and back-crossing the resistant rice with other rice germplasm through standard means will yield herbicide-resistant rice varieties and hybrids having good productivity and other agronomically desirable properties.

Because red rice and commercial rice belong to the same species, the planting of a herbicide-resistant commercial rice crop entails some risk that herbicide resistance would be transferred to red rice. However, rice is self-pollinating, and the frequency of outcrossing is low, even between immediately adjacent plants flowering in synchrony. The likelihood of transferring resistance to red rice could be minimized by breeding resistant varieties that flower significantly earlier than does red rice (e.g., using conventional breeding techniques, or by tissue culture such as anther culture). Maintaining an early-maturing phenotype in resistant varieties, for example, will be desirable to reduce the likelihood of outcrossing to red rice. In addition, breeding higher levels of resistance (e.g., by crossing lines with different AHAS isozymes with one another, or crossing lines with resistant AHAS enzymes with the metabolic resistance of ATCC 75295) will allow control of the outcrossed red rice by applying higher herbicide rates than the outcrossed red rice will tolerate.

If a strain of red rice should nevertheless develop that is resistant to the same herbicides as the resistant commercial rice, the plants can always be treated with a broad range of other available herbicides -- particularly if the resistant red rice were discovered early, before having much opportunity to propagate.

Because each of the herbicides tested inhibits the activity of acetohydroxyacid synthase, and because resistance to each of these herbicides has been demonstrated in the novel lines, it is expected that the novel herbicide resistant rice will show resistance to other herbicides that

5 normally inhibit this enzyme. In addition to those discussed above, such herbicides include
others of the imidazolinone and sulfonylurea classes, including at least the following:
10 primisulfuron, chlorsulfuron, imazamethabenz methyl, and triasulfuron. Other classes of
AHAS herbicides known in the art include triazolopyrimidines, sulfamoylureas,
5 sulfonylcarboxamides, sulfonamides, pyrimidylloxybenzoates, phthalides, pyrimidylsalicylates,
carbamoylpyrazolines, sulfonylimino-triazinyl heteroazoles, N-protected valylanilides,
sulfonylamide azines, pyrimidyl maleic acids, benzenesulfonyl carboxamides, substituted
15 sulfonyldiamides, and ubiquinone-o.

10 As used in the specification and claims, the term "mutation-inducing conditions" refers
to conditions that will cause mutations in a plant's genome at rates substantially higher than the
20 background rate. A variety of such conditions are well-known to those in the art. They
include, for example, exposing seeds to chemical mutagens or ionizing radiation as described
above. Such conditions also include growing cells in tissue culture (anther culture, callus
25 culture, suspension culture, protoplast culture, etc.), with or without deliberately exposing the
cells to additional mutation-inducing conditions other than those that are inherent in tissue
culture. (It is known that tissue culture is *per se* conducive to the production of genetic
variability, including mutations.) Depending on the particular mutation-inducing conditions
30 used, mutations may best be induced at different stages in the life cycle, e.g., with dry seeds,
20 with pre-germinated seeds, etc.

35 As used in the specification and claims, the term "imidazolinone" means a herbicidal
composition comprising one or more chemical compounds of the imidazolinone class, including
by way of example and not limitation, 2-(2-imidazolin-2-yl)pyridines,
25 2-(2-imidazolin-2-yl)quinolines and 2-(2-imidazolin-2-yl) benzoates or derivatives thereof,
including their optical isomers, diastereomers and/or tautomers exhibiting herbicidal activity,
40 including by way of example and not limitation
2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3- quinolinecarboxylic acid
(generic name imazaquin); 2-[4,5-dihydro-4-methyl-4-
45 (1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid (generic name
imazethapyr); and 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-
(methoxymethyl)-3-pyridinecarboxylic acid (generic name imazamox); 2-[4,5-dihydro-4-methyl-
50 4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid (generic name imazapyr);
2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-

pyridinecarboxylic acid) (generic name imazameth, also known as imazapic); and the other examples of imidazolinone herbicides given in the specification.

As used in the specification and claims, the term "sulfonyleurea" means a herbicidal composition comprising one or more chemical compounds of the sulfonyleurea class, which generally comprise a sulfonyleurea bridge, $-\text{SO}_2\text{NHCONH}-$, linking two aromatic or heteroaromatic rings, including by way of example and not limitation 2-((((4,6-dimethoxypyrimidin-2-yl) aminocarbonyl)) aminosulfonyl))-N,N-dimethyl-3-pyridinecarboxamide (generic name nicosulfuron); 3-[4,6-bis (difluoromethoxy)-pyrimidin-2-yl]-1-(2-methoxycarbonylphenylsulfonyl) urea (generic name primisulfuron); 2-((((4,6-dimethyl-2-pyrimidinyl)amino)carbonyl)amino)sulfonyl]benzoic acid methyl ester (generic name sulfometuron methyl); methyl 2-((((4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino)carbonyl)amino)sulfonyl]benzoate (generic name metsulfuron methyl); methyl-2-((((4-methoxy-6-methyl-1,3,5-triazin-2-yl) methylamino)carbonyl)amino)sulfonyl]benzoate (generic name tribenuron methyl); methyl-3-((((4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino)carbonyl)amino)sulfonyl]-2-thiophenecarboxylate (generic name thifensulfuron methyl); 2-chloro-N-((4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl)benzenesulfonamide (generic name chlorsulfuron); ethyl 2-((((4-chloro-6-methoxypyrimidin-2-yl)amino)carbonyl)amino)sulfonyl benzoate (generic name chlorimuron ethyl); methyl 2-((((4-methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino)carbonyl)amino)sulfonyl benzoate (generic name tribenuron methyl); 3-(6-methoxy-4-methyl-1,3,5-triazin-2-yl)-1-[2-(2-chloroethoxy)-phenylsulfonyl]-urea (generic name triasulfuron); and the other examples of sulfonyleurea herbicides given in the specification.

As used in the specification and claims, unless otherwise clearly indicated by context, the term "plant" is intended to encompass plants at any stage of maturity, as well as any cells, tissues, or organs taken or derived from any such plant, including without limitation any seeds, leaves, stems, flowers, fruits, roots, tubers, single cells, gametes, anther cultures, callus cultures, suspension cultures, other tissue cultures, or protoplasts.

Unless otherwise clearly indicated by context, the "progeny" of a plant includes a plant of any subsequent generation whose ancestry can be traced to that plant.

Unless otherwise clearly indicated by context, a "derivative" of a herbicide-resistant plant includes both the progeny of that herbicide-resistant plant, as the term "progeny" is defined above; and also any mutant, recombinant, or genetically-engineered derivative of that

5 plant, whether of the same species or of a different species; where, in either case, the herbicide-resistance characteristics of the original herbicide-resistant plant have been transferred to the derivative plant. Thus a "derivative" of a rice plant with a resistant AHAS enzyme would include, by way of example and not limitation, any of the following plants that
10 express the same resistant AHAS enzyme: F₁ progeny rice plants, F₂ progeny rice plants, and F₃₀ progeny rice plants.

15 The following definitions should be understood to apply throughout the specification and claims. A "functional" or "normal" AHAS enzyme is one that is capable of catalyzing the first step in the pathway for synthesis of the essential amino acids isoleucine, leucine, and
20 valine. A "wild-type" AHAS enzyme or "wild-type" AHAS sequence is an AHAS enzyme or a DNA sequence encoding an AHAS enzyme, respectively, that is present in a herbicide-sensitive member of a given species. A "resistant" plant is one that produces a functional AHAS enzyme, and that is capable of reaching maturity when grown in the presence of normally inhibitory levels of a herbicide that normally inhibits AHAS. The term "resistant" or
25 "resistance," as used herein, is also intended to encompass "tolerant" plants, i.e., those plants that phenotypically evidence adverse, but not lethal, reactions to one or more AHAS herbicides. A "resistant" AHAS enzyme is a functional AHAS enzyme that retains substantially greater activity than does a wild-type AHAS enzyme in the presence of normally inhibitory levels of an AHAS herbicide, as measured by *in vitro* assays of the respective
30 enzymes' activities. A "wild-type" or "sensitive" plant is one that produces a functional AHAS enzyme, and that is sensitive to normally inhibitory levels of a herbicide that normally inhibits AHAS. Note that within the contemplation of this last definition, "wild-type" plants include cultivated varieties; the designation "wild-type" refers to the presence or absence of normal
35 levels of herbicide sensitivity, and in the context of this specification and the claims the term "wild-type" carries no connotation as to whether a particular plant is the product of cultivation and artificial selection, or is found in nature in an uncultivated state.

40 The complete disclosures of all references cited in this specification are hereby incorporated by reference, as is the complete disclosure of United States provisional patent application serial number 60/107,255, filed November 5, 1998. In the event of an otherwise irreconcilable conflict, however, the present specification shall control.

50 Notes on herbicide nomenclature -- the following listing gives trade names, generic names, and chemical names for various herbicides: Pursuit™ (imazethapyr: (±)-2-[4,5-

dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic
 acid); Scepter™ (imazaquin: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-
 yl]-3-quinolinecarboxylic acid); Accent™ (nicosulfuron: 2-((((4,6-dimethoxypyrimidin-2-yl)
 aminocarbonyl)) aminosulfonyl))-N,N-dimethyl-3-pyridinecarboxamide); Beacon™
 (primisulfuron: 3-[4,6-bis (difluoromethoxy)-pyrimidin-2-yl]-1-(2-
 methoxycarbonylphenylsulfonyl) urea); Raptor™ (imazamox: (+)-5-methoxymethyl-2-(4-
 isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) nicotinic acid; Cadre™ (imazapic: (±)-2-[4,5-
 dihydro-4-methyl-4-(1-methyl-ethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic
 acid; alternate chemical name (±)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-
 methylnicotinic acid); Arsenal™ (imazapyr: 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-
 1H-imidazol-2-yl]-3-pyridinecarboxylic acid); Oust™ (sulfometuron methyl: chemical name 2-
 [[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid methyl ester);
 Ally™ (metsulfuron methyl: methyl 2-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-
 yl)amino]carbonyl]amino]sulfonyl]benzoate); Harmony™ (mixture of thifensulfuron methyl and
 tribenuron methyl: mixture of methyl-3-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)
 amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate and methyl-2-[[[N-(4-methoxy-6-
 methyl-1,3,5-triazin-2-yl) methylamino]carbonyl]amino]sulfonyl]benzoate); Pinnacle™
 (thifensulfuron methyl: methyl-3-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)
 amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate); Glean™ or Telar™ (chlorsulfuron:
 2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide);
 Classic™ (chlorimuron ethyl: ethyl 2-[[[(4-chloro-6-methoxypyrimidin-2-
 yl)amino]carbonyl]amino]sulfonyl benzoate); Express™ (tribenuron methyl: methyl 2-[[[N-(4-
 methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino]carbonyl]amino]sulfonyl benzoate); Assert™
 (imazamethabenz methyl: *m*-toluic acid, 6-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-,
 methyl ester; and *p*-toluic acid, 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-, methyl
 ester); and Amber™ (triasulfuron: 3-(6-methoxy-4-methyl-1,3,5-triazin-2-yl)-1-[2-(2-
 chloroethoxy)-phenylsulfonyl]-urea); Staple™ (pyrithiobac sodium: sodium 2-chloro-6-[(4,6-
 dimethoxy pyrimidin-2-yl)thio]benzoate); and Matrix™ (rimsulfuron: N-((4,6-
 dimethoxypyrimidin-2-yl)aminocarbonyl)-3-(ethylsulfonyl)-2-pyridinesulfonamide).

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 136a)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>11</u> , line <u>7-12</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 02 November 1999	Accession Number (Not yet known. Applicant's designation PWC18.)
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer STEFAN POSTAD INTERNATIONAL BUREAU OF PATENT CO-OPERATION 703-3095-5000	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer

PCT/US99/26062 (Ind. 1999)

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>11</u> , line <u>7-12</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 02 November 1999	Accession Number (Not yet known. Applicant's designation PWC23.)
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<input checked="" type="checkbox"/> For receiving Office use only This sheet was received with the international application Authorized officer 703-3000000	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on: Authorized officer

PCT/US/112 (July 1998)

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>11</u> , line <u>7-12</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 02 November 1999	Accession Number (Not yet known. Applicant's designation CMC29.)
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer <u>SIGEL</u> DATE <u>10/2</u>	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer

PCT/US/99/26062 (July 1998)

Applicant's or agent's file reference	International application No. 98A8-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 136a)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>11</u> , line <u>7-12</u>	
B. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 02 November 1999	Accession Number (Not yet known. Applicant's designation CMC31.)
C. ADDITIONAL INDICATIONS (leave blank if not applicable)	
This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	
<input checked="" type="checkbox"/> This sheet was received with the international application	
Authorized officer	
For International Bureau use only	
<input type="checkbox"/> This sheet was received by the International Bureau on:	
Authorized officer	

PCT/US99/26062 (Rev. 1992)

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>11</u> , line <u>7-12</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 02 November 1999	Accession Number (Not yet known. Applicant's designation WDC33.)
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<input checked="" type="checkbox"/> For receiving Office use only This sheet was received with the international application	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)


A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>11</u> , line <u>7-12</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 02 November 1999	Accession Number (Not yet known. Applicant's designation WDC37.)
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div>For receiving Office use only</div> <div><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div>Authorized officer</div>	<div>For International Bureau use only</div> <div><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div>Authorized officer</div>

PCT/US99/26062 (Indication)

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13b1a)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>11</u> , line <u>7-12</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 02 November 1999	Accession Number (Not yet known. Applicant's designation WDC38.)
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (If the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<input checked="" type="checkbox"/> For receiving Office use only This sheet was received with the international application Authorized officer 	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on: Authorized officer

PCT/US99/26062 (July 1998)

5

Applicant's or agent's file reference	International application No. 98A8-PCT
--	---

10

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 136is)

15

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description
on page 9, line 28-35

20

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

American Type Culture Collection

Address of depositary institution (including postal code and country)

10801 University Boulevard
Manassas, VA 20110-2209
United States of America

25

Date of deposit

05 November 1998

Accession Number

203419

30

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet ☐

35

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

40

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession
Number of Deposit")

45

For receiving Office use only

☒ This sheet was received with the international application

Authorized officer

For International Bureau use only

☐ This sheet was received by the International Bureau on:

Authorized officer

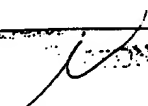
50

55

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>9</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203420
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<input checked="" type="checkbox"/> For receiving Office use only This sheet was received with the international application Authorized officer: 	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on: Authorized officer:

Form PCT/PC/VI 14 (July 1998)

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>9</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203421
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div>For receiving Office use only</div> <div><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div>Authorized officer</div>	<div>For International Bureau use only</div> <div><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div>Authorized officer</div>

Form PCT/DC/114 (July 1998)

5

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

10

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

15

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>9</u> , line <u>28-35</u>	
---	--

20

B. IDENTIFICATION OF DEPOSIT	Further deposits are identified on an additional sheet <input type="checkbox"/>
------------------------------	---

Name of depositary institution
American Type Culture Collection

Address of depositary institution (including postal code and country)

10801 University Boulevard
Manassas, VA 20110-2209
United States of America

25

Date of deposit 05 November 1998	Accession Number 203422
-------------------------------------	----------------------------

C. ADDITIONAL INDICATIONS (leave blank if not applicable)	This information is continued on an additional sheet <input type="checkbox"/>
---	---

30

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

35

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

45

<p>For receiving Office use only</p> <p><input checked="" type="checkbox"/> This sheet was received with the international application</p> <p>Authorized officer 703.305</p>	<p>For International Bureau use only</p> <p><input type="checkbox"/> This sheet was received by the International Bureau on:</p> <p>Authorized officer</p>
--	--

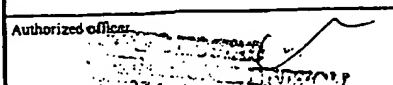
50

55

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

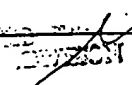
A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>9</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203423
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer 	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer

PCT/US99/26062

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>9</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203424
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer: 	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer:

PCT/US99/26062 (11/1/1998)

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>9</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203425
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer SIGNED FOR HEAD INTERNATIONAL DIVISION 703.505.300	Authorized officer

PCT/US/99/26062 (Rev. 10/98)

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL(PCT Rule 13*bis*)

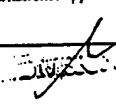
A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>8</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203428
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<div>For receiving Office use only</div> <div><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div>Authorized officer: INTER. DIVISION 203,205-3980</div>	<div>For International Bureau use only</div> <div><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div>Authorized officer:</div>

PCT/US/99/26062 (July 1999)

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 136b)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>28-35</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT	
Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203427
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<input checked="" type="checkbox"/> For receiving Office use only This sheet was received with the international application Authorized officer: 	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on: Authorized officer:

Form PCT/IS/136 (July 1998)

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL(PCT Rule 136*is*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>9</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203428
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	
For receiving Office use only	
<input checked="" type="checkbox"/> This sheet was received with the international application	
Authorized officer STAD INTERNATIONAL DIVISION 703-305-3680	
For International Bureau use only	
<input type="checkbox"/> This sheet was received by the International Bureau on:	
Authorized officer	

Applicant's or agent's file reference	International application No. 88A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>9</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203429
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer: 203 505 5400 203 505 5400	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer:

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>9</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203430
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only <input checked="" type="checkbox"/> This sheet was received with the international application Authorized officer 703-305-4800	For International Bureau use only <input type="checkbox"/> This sheet was received by the International Bureau on: Authorized officer

Form PCT/IB/13bis (July 1998)

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>8</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203431
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<input checked="" type="checkbox"/> For receiving Office use only This sheet was received with the international application Authorized officer [Signature] [Stamp: RECEIVED 17-NOV-1998]	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on: Authorized officer

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>9</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1988	Accession Number 203432
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
For receiving Office use only	For International Bureau use only
<input checked="" type="checkbox"/> This sheet was received with the international application	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

PCT/US/112 (July 1992)

Applicant's or agent's file reference	International application No. 98A9-PCT
--	---

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page <u>9</u> , line <u>28-35</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, VA 20110-2209 United States of America	
Date of deposit 05 November 1998	Accession Number 203433
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<input checked="" type="checkbox"/> For receiving Office use only This sheet was received with the international application	<input type="checkbox"/> For International Bureau use only This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

PCT/US/13a (July 1998)

Claims

5

10

15

20

25

30

35

40

45

50

55

What is claimed:

1. A rice plant wherein:

(a) the growth of said plant is resistant to inhibition by one or more of the following herbicides, at levels of herbicide that would normally inhibit the growth of a rice plant: imazethapyr, imazapic, imazapyr, nicosulfuron, sulfometuron methyl, imazaquin, imazamox, chlorimuron ethyl, metsulfuron methyl, rimsulfuron, thifensulfuron methyl, tribenuron methyl, pyriithiobac sodium, or a derivative of any of these herbicides; and

(b) said plant is a derivative of at least one of the plants selected from the group of plants with ATCC accession numbers 203419, 203420, 203421, 203422, 203423, 203424, 203425, 203426, 203427, 203428, 203429, 203430, 203431, 203432, 203433, aaaaa, bbbbbb, cccccc, ddddd, eeeee, fffff, and ggggg; and

(c) said plant has the herbicide resistance characteristics of at least one of the plants selected from the group of plants with ATCC accession numbers 203419, 203420, 203421, 203422, 203423, 203424, 203425, 203426, 203427, 203428, 203429, 203430, 203431, 203432, 203433, aaaaa, bbbbbb, cccccc, ddddd, eeeee, fffff, and ggggg.

2. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to inhibition by imazethapyr, at levels of imazethapyr that would normally inhibit the growth of a rice plant.

3. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to inhibition by imazapic, at levels of imazapic that would normally inhibit the growth of a rice plant.

4. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to inhibition by imazapyr, at levels of imazapyr that would normally inhibit the growth of a rice plant.

5

1 5. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to
2 inhibition by nicosulfuron, at levels of nicosulfuron that would normally inhibit the growth of a
rice plant.

10

1 6. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to
2 inhibition by sulfometuron methyl, at levels of sulfometuron methyl that would normally inhibit
3 the growth of a rice plant.

15

4 7. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to
5 inhibition by imazaquin, at levels of imazaquin that would normally inhibit the growth of a rice
plant.

20

1 8. A rice plant as recited in Claim 1, wherein the growth of said plant is additionally
2 resistant to inhibition by primisulfuron, at levels of primisulfuron that would normally inhibit
the growth of a rice plant.

25

1 9. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to
2 inhibition by imazamox, at levels of imazamox that would normally inhibit the growth of a rice
plant.

30

1 10. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to
2 inhibition by chlorimuron ethyl, at levels of chlorimuron ethyl that would normally inhibit the
growth of a rice plant.

35

1 11. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to
2 inhibition by metsulfuron methyl, at levels of metsulfuron methyl that would normally inhibit
the growth of a rice plant.

40

1 12. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to
2 inhibition by rimsulfuron, at levels of rimsulfuron that would normally inhibit the growth of a
rice plant.

45

1 13. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to
2 inhibition by thifensulfuron methyl, at levels of thifensulfuron methyl that would normally
inhibit the growth of a rice plant.

50

55

5

1 14. A rice plant as recited in Claim 1, wherein the growth of said plant is additionally
2 resistant to inhibition by tribenuron methyl, at levels of tribenuron methyl that would normally
inhibit the growth of a rice plant.

10

1 15. A rice plant as recited in Claim 1, wherein the growth of said plant is resistant to
2 inhibition by pyriithiobac sodium, at levels of pyriithiobac sodium that would normally inhibit
the growth of a rice plant.

15

1 16. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203419, or is any progeny of the plant with ATCC accession number 203419; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
4 203419.

20

1 17. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203420, or is any progeny of the plant with ATCC accession number 203420; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
203420.

25

1 18. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203421, or is any progeny of the plant with ATCC accession number 203421; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
203421.

30

1 19. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203422, or is any progeny of the plant with ATCC accession number 203422; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
203422.

35

1 20. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203423, or is any progeny of the plant with ATCC accession number 203423; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
203423.

40

45

50

55

5
1 21. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203424, or is any progeny of the plant with ATCC accession number 203424; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
10 203424.

1 22. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203425, or is any progeny of the plant with ATCC accession number 203425; wherein
15 3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
203425.

1 23. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
20 2 number 203426, or is any progeny of the plant with ATCC accession number 203426; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
203426.

25 1 24. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203427, or is any progeny of the plant with ATCC accession number 203427; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
30 203427.

1 25. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203428, or is any progeny of the plant with ATCC accession number 203428; wherein
35 3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
203428.

1 26. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
40 2 number 203429, or is any progeny of the plant with ATCC accession number 203429; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
203429.

45 1 27. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203430, or is any progeny of the plant with ATCC accession number 203430; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
50 203430.

5

1 28. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203431, or is any progeny of the plant with ATCC accession number 203431; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
10 203431.

15

1 29. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203432, or is any progeny of the plant with ATCC accession number 203432; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
203432.

20

1 30. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number 203433, or is any progeny of the plant with ATCC accession number 203433; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
203433.

25

1 31. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number aaaaa, or is any progeny of the plant with ATCC accession number aaaaa; wherein said
3 plant has the herbicide resistance characteristics of the plant with ATCC accession number
30 4 aaaaa.

35

1 32. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number bbbbb, or is any progeny of the plant with ATCC accession number bbbbb; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
4 bbbbb.

40

1 33. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number ccccc, or is any progeny of the plant with ATCC accession number ccccc; wherein said
3 plant has the herbicide resistance characteristics of the plant with ATCC accession number
4 ccccc.

45

1 34. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number ddddd, or is any progeny of the plant with ATCC accession number ddddd; wherein
3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
50 4 ddddd.

55

5
1 35. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number eeeee, or is any progeny of the plant with ATCC accession number eeeee; wherein said
3 plant has the herbicide resistance characteristics of the plant with ATCC accession number
10 4 eeeee.

1 36. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number fffff, or is any progeny of the plant with ATCC accession number fffff; wherein said
15 3 plant has the herbicide resistance characteristics of the plant with ATCC accession number
4 fffff.

1 37. A rice plant as recited in Claim 1, wherein said plant is the plant with ATCC accession
2 number ggggg, or is any progeny of the plant with ATCC accession number ggggg; wherein
20 3 said plant has the herbicide resistance characteristics of the plant with ATCC accession number
4 ggggg.

25 5 38. A process for controlling weeds in the vicinity of a rice plant as recited in Claim 1,
6 said process comprising applying a herbicide to the weeds and to the rice plant, wherein the
7 herbicide comprises imazethapyr, imazapic, imazapyr, nicosulfuron, sulfometuron methyl,
30 8 imazaquin, primisulfuron, imazamox, chlorimuron ethyl, metsulfuron methyl, rimsulfuron,
9 thifensulfuron methyl, tribenuron methyl, pyriithiobac sodium, or a derivative of any of these
herbicides.

35 1 39. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203419, or is any progeny of the plant with ATCC accession number 203419; wherein
3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
40 203419.

1 40. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203420, or is any progeny of the plant with ATCC accession number 203420; wherein
45 3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
203420.

50

55

5

1 41. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203421, or is any progeny of the plant with ATCC accession number 203421; wherein
3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
10 203421.

10

1 42. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203422, or is any progeny of the plant with ATCC accession number 203422; wherein
15 3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
203422.

15

1 43. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203423, or is any progeny of the plant with ATCC accession number 203423; wherein
20 3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
203423.

20

1 44. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203424, or is any progeny of the plant with ATCC accession number 203424; wherein
25 3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
30 203424.

25

1 45. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203425, or is any progeny of the plant with ATCC accession number 203425; wherein
35 3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
203425.

35

1 46. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203426, or is any progeny of the plant with ATCC accession number 203426; wherein
40 3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
203426.

40

1 47. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203427, or is any progeny of the plant with ATCC accession number 203427; wherein
45 3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
50 203427.

45

50

55

5

1 48. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203428, or is any progeny of the plant with ATCC accession number 203428; wherein
3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
10 203428.

15

1 49. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203429, or is any progeny of the plant with ATCC accession number 203429; wherein
3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
203429.

20

1 50. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203430, or is any progeny of the plant with ATCC accession number 203430; wherein
3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
203430.

25

1 51. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203431, or is any progeny of the plant with ATCC accession number 203431; wherein
3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
30 203431.

35

1 52. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203432, or is any progeny of the plant with ATCC accession number 203432; wherein
3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
203432.

40

1 53. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number 203433, or is any progeny of the plant with ATCC accession number 203433; wherein
3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
4 203433.

45

1 54. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number aaaaa, or is any progeny of the plant with ATCC accession number aaaaa; wherein the
3 plant has the herbicide resistance characteristics of the plant with ATCC accession number
4 aaaaa.

55

5

1 55. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number bbbbb, or is any progeny of the plant with ATCC accession number bbbbb; wherein
10 3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
4 bbbbb.

15

1 56. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number ccccc, or is any progeny of the plant with ATCC accession number ccccc; wherein the
3 plant has the herbicide resistance characteristics of the plant with ATCC accession number
4 ccccc.

20

1 57. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number ddddd, or is any progeny of the plant with ATCC accession number ddddd; wherein
3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
25 4 ddddd.

25

1 58. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number eeeee, or is any progeny of the plant with ATCC accession number eeeee; wherein the
30 3 plant has the herbicide resistance characteristics of the plant with ATCC accession number
4 eeeee.

30

1 59. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number fffff, or is any progeny of the plant with ATCC accession number fffff; wherein the
35 3 plant has the herbicide resistance characteristics of the plant with ATCC accession number
4 fffff.

35

1 60. A process as recited in Claim 38, wherein the plant is the plant with ATCC accession
2 number ggggg, or is any progeny of the plant with ATCC accession number ggggg; wherein
40 3 the plant has the herbicide resistance characteristics of the plant with ATCC accession number
45 ggggg

40

1 61. A process for controlling weeds in the vicinity of a rice plant as recited in Claim 1,
2 said process comprising applying a herbicide to the weeds and to the rice plant, wherein the
50 3 herbicide comprises primisulfuron, triasulfuron, chlorsulfuron, imazamethabenz methyl, or a
derivative of any of these herbicides.

50

55

5

1 **62.** A herbicide-resistant rice plant, wherein:

10

2 (a) the growth of said herbicide-resistant plant is resistant to inhibition by at least one
3 herbicide that normally inhibits acetohydroxyacid synthase, at levels of the herbicide
4 that would normally inhibit the growth of a rice plant; and

15

5 (b) said herbicide-resistant plant is a derivative of a rice plant obtained by exposing rice
6 plants to mutation-inducing conditions; growing rice plants from the exposed plants, or
7 growing rice plants from progeny of the exposed plants, in the presence of at least one
8 herbicide that normally inhibits acetohydroxyacid synthase, at levels of the herbicide
9 that would normally inhibit the growth of a rice plant; and selecting for further
10 propagation rice plants that grow without significant injury in the presence of the
11 herbicide; and

25

12 (c) said herbicide-resistant plant expresses a functional acetohydroxyacid synthase that
13 is resistant to inhibition by at least one herbicide that normally inhibits
14 acetohydroxyacid synthase, at levels of the herbicide that would normally inhibit the
15 growth of a rice plant;

30

16 *provided that excluded from the scope of this Claim is:*

35

17 (d) a plant that is the plant with ATCC accession number 97523; and any mutant,
18 recombinant, or genetically engineered derivative of the plant with ATCC accession
19 number 97523 or of any progeny of the plant with ATCC accession number 97523; and
20 any plant that is the progeny of any of these plants; wherein these derivatives of the
21 plant with ATCC accession number 97523 that are excluded from the scope of this
22 Claim are those that retain the herbicide resistance characteristics of the plant with
 ATCC accession number 97523.

40

45

1 **63.** A process for controlling weeds in the vicinity of a rice plant as recited in Claim 62,
2 said process comprising applying a herbicide to the weeds and to the rice plant, wherein the
3 herbicide normally inhibits acetohydroxyacid synthase, at levels of the herbicide that would
 normally inhibit the growth of a rice plant.

50

55

5

1 64. A rice plant as recited in Claim 62, wherein the growth of said plant is resistant to
2 inhibition by at least one imidazolinone herbicide that normally inhibits acetohydroxyacid
synthase, at levels of the herbicide that would normally inhibit the growth of a rice plant.

10

1 65. A process for controlling weeds in the vicinity of a rice plant as recited in Claim 64,
2 said process comprising applying an imidazolinone herbicide to the weeds and to the rice plant,
3 wherein the herbicide normally inhibits acetohydroxyacid synthase, at levels of the herbicide
that would normally inhibit the growth of a rice plant.

15

1 66. A rice plant as recited in Claim 62, wherein the growth of said plant is resistant to
2 inhibition by at least one sulfonyleurea herbicide that normally inhibits acetohydroxyacid
synthase, at levels of the herbicide that would normally inhibit the growth of a rice plant.

20

1 67. A process for controlling weeds in the vicinity of a rice plant as recited in Claim 66,
2 said process comprising applying a sulfonyleurea herbicide to the weeds and to the rice plant,
3 wherein the herbicide normally inhibits acetohydroxyacid synthase, at levels of the herbicide
that would normally inhibit the growth of a rice plant.

25

1 68. A rice plant as recited in Claim 62, wherein the growth of said plant is resistant to
2 inhibition by at least one herbicide selected from the group consisting of imazethapyr,
3 imazapic, imazapyr, nicosulfuron, sulfometuron methyl, imazaquin, primisulfuron, imazamox,
4 chlorimuron ethyl, metsulfuron methyl, rimsulfuron, thifensulfuron methyl, tribenuron methyl,
5 and pyriithiobac sodium; at levels of the herbicide that would normally inhibit the growth of a
rice plant.

30

40

45

50

55

5

1 69. A process for controlling weeds in the vicinity of a rice plant as recited in Claim 68,
2 said process comprising applying to the weeds and to the rice plant at least one herbicide
3 selected from the group consisting of imazethapyr, imazapic, imazapyr, nicosulfuron,
4 sulfometuron methyl, imazaquin, primisulfuron, imazamox, chlorimuron ethyl, metsulfuron
5 methyl, rimsulfuron, thifensulfuron methyl, tribenuron methyl, and pyrithiobac sodium; at
levels of the herbicide that would normally inhibit the growth of a rice plant.

15

1 70. A rice plant as recited in Claim 62, wherein the mutation-inducing conditions comprise
exposing rice seeds to a mutagen.

20

1 71. A process for imparting herbicide resistance to rice plants, said process comprising the
2 steps of:

25

3 (a) exposing rice plants to mutation-inducing conditions;

30

4 (b) growing rice plants from the exposed plants, or growing rice plants from progeny
5 of the exposed plants, in the presence of at least one herbicide that normally inhibits
6 acetohydroxyacid synthase, at levels of the herbicide that would normally inhibit the
7 growth of a rice plant; and

35

8 (c) selecting for further propagation rice plants that grow without significant injury in
9 the presence of the herbicide.

40

1 72. A process as recited in Claim 71, wherein the herbicide is selected from the group
consisting of imazethapyr, imazapic, and imazapyr.

1 73. A process as recited in Claim 71, wherein said exposing step comprises exposing rice
seeds to a mutagen.

45

1 74. A process as recited in Claim 71, wherein the plants selected for further propagation
2 express a functional acetohydroxyacid synthase that is resistant to inhibition by at least one
3 herbicide that normally inhibits acetohydroxyacid synthase, at levels of the herbicide that would
normally inhibit the growth of a rice plant.

50

55

5

1 **75.** A herbicide-resistant rice plant, wherein:

10

2 (a) the growth of said herbicide-resistant plant is resistant to inhibition by at least one
3 herbicide that normally inhibits acetohydroxyacid synthase, at levels of the herbicide
4 that would normally inhibit the growth of a rice plant;

15

5 (b) said herbicide-resistant plant expresses functional first and second resistant
6 acetohydroxyacid synthases, each of which said resistant acetohydroxyacid synthases is
7 resistant to inhibition by at least one herbicide that normally inhibits acetohydroxyacid
8 synthase, at levels of the herbicide that would normally inhibit the growth of a rice
9 plant;

20

10 (c) said first and second resistant acetohydroxyacid synthases are not identical; and

25

11 (d) said first resistant acetohydroxyacid synthase is a mutated form of a first wild-type
12 rice acetohydroxyacid synthase; and said second resistant acetohydroxyacid synthase is
13 a mutated form of a second wild-type rice acetohydroxyacid synthase; wherein the first
14 and second wild-type rice acetohydroxyacid synthases are different enzymes that are
30 normally encoded by different genes of wild-type rice plants.

30

1 **76.** A process for controlling weeds in the vicinity of a rice plant as recited in Claim 75,
35 2 said process comprising applying a herbicide to the weeds and to the rice plant, wherein the
3 herbicide normally inhibits acetohydroxyacid synthase, at levels of the herbicide that would
 normally inhibit the growth of a rice plant.

35

1 **77.** A rice plant as recited in Claim 75, wherein the growth of said plant is resistant to
40 2 inhibition by at least one imidazolinone herbicide that normally inhibits acetohydroxyacid
 synthase, at levels of the herbicide that would normally inhibit the growth of a rice plant.

40

1 **78.** A process for controlling weeds in the vicinity of a rice plant as recited in Claim 77,
45 2 said process comprising applying an imidazolinone herbicide to the weeds and to the rice plant,
3 wherein the herbicide normally inhibits acetohydroxyacid synthase, at levels of the herbicide
50 that would normally inhibit the growth of a rice plant.

45

50

55

5

1 79. A rice plant as recited in Claim 75, wherein the growth of said plant is resistant to
2 inhibition by at least one sulfonylurea herbicide that normally inhibits acetohydroxyacid
synthase, at levels of the herbicide that would normally inhibit the growth of a rice plant.

10

1 80. A process for controlling weeds in the vicinity of a rice plant as recited in Claim 79,
2 said process comprising applying a sulfonylurea herbicide to the weeds and to the rice plant,
3 wherein the herbicide normally inhibits acetohydroxyacid synthase, at levels of the herbicide
that would normally inhibit the growth of a rice plant.

15

1 81. A rice plant as recited in Claim 75, wherein said plant is a derivative of the plant with
2 ATCC accession number 75295, and said plant additionally has the herbicide resistance
characteristics of the plant with ATCC accession number 75295.

20

25

30

35

40

45

50

55

INTERNATIONAL SEARCH REPORT

Inventor and Applicant No.
PCT/US 99/26062

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01H5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data bases consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 154 204 A (MOLECULAR GENETICS INC) 11 September 1985 (1985-09-11) claims 7-9	62, 64, 66, 68
X	& US 5 718 079 A (ANDERSON ET AL.) 17 February 1998 (1998-02-17) cited in the application column 9, line 5 - line 40	71-74
X	US 5 736 629 A (CROUGHAN TIMOTHY P) 7 April 1998 (1998-04-07) cited in the application	62-74
A	the whole document	1, 38, 61, 75, 76, 80
A	US 5 545 822 A (CROUGHAN TIMOTHY P) 13 August 1996 (1996-08-13) cited in the application the whole document	1, 38, 61-74
-/-		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document relating to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

29 February 2000

Date of mailing of the international search report

07/03/2000

Name and mailing address of the ISA

European Patent Office, P.B. 6318 Patendree 8
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 631 cpo nl,
Fax (+31-70) 340-3016

Authorized officer

Fonts Cavestany, A

Form PCT/ISA/210 (second sheet) (July 1993)

INTERNATIONAL SEARCH REPORT

Date of Application No.
PCT/US 99/26062

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 290 753 A (NEEHOUSE KEITH E ET AL) 1 March 1994 (1994-03-01) column 2, line 28; claims	1,38, 61-74
A	US 5 773 704 A (CROUGHAN TIMOTHY P) 30 June 1998 (1998-06-30) cited in the application column 8, line 46 -column 10, line 50; claims	75-81
A	US 5 605 011 A (CHALEFF ROY S ET AL) 25 February 1997 (1997-02-25) column 27, line 12; claims 1,2	1,38, 61-74

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inventor: not Applicable to

PCT/US 99/26062

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0154204 A	11-09-1985	AT 100141 T	15-01-1994
		AU 3950785 A	12-09-1985
		AU 4494293 A	18-11-1993
		AU 642633 B	28-10-1993
		AU 4608989 A	29-03-1990
		AU 6192396 A	31-10-1996
		BR 8500986 A	29-10-1985
		DE 3587718 D	24-02-1994
		DE 3587718 T	04-08-1994
		JP 60210929 A	23-10-1985
		US 5718079 A	17-02-1998
		US 5331107 A	19-07-1994
		US 5304732 A	19-04-1994
		US 4761373 A	02-08-1988
US 5736629 A	07-04-1998	US 5773704 A	30-06-1998
		AU 2993197 A	19-11-1997
		CN 1230221 A	29-09-1999
		EP 0914420 A	12-05-1999
		US 5952553 A	14-09-1999
		WO 9741218 A	06-11-1997
US 5545822 A	13-08-1996	US 5773703 A	30-06-1998
US 5290753 A	01-03-1994	AT 154390 T	15-06-1997
		AU 641496 B	23-09-1993
		AU 7605891 A	14-11-1991
		CA 2041728 A	05-11-1991
		DE 69126474 D	17-07-1997
		DE 69126474 T	11-12-1997
		DK 461355 T	25-08-1997
		EP 0461355 A	18-12-1991
		ES 2104628 T	16-10-1997
		GR 3023764 T	30-09-1997
		PT 97590 A, B	28-02-1992
		ZA 9103510 A	26-02-1992
US 5773704 A	30-06-1998	AU 2993197 A	19-11-1997
		CN 1230221 A	29-09-1999
		EP 0914420 A	12-05-1999
		US 5952553 A	14-09-1999
		WO 9741218 A	06-11-1997
		US 5736629 A	07-04-1998
US 5605011 A	25-02-1997	US 5378824 A	03-01-1995
		US 5013659 A	07-05-1991
		US 5141870 A	25-08-1995
		AT 145648 T	15-12-1996
		AU 619167 B	23-01-1992
		AU 7638387 A	03-03-1988
		CA 1314506 A	16-03-1993
		CN 1040024 B	30-09-1998
		DE 3751963 D	09-01-1997
		DE 3751963 T	28-05-1997
		DK 443187 A	27-02-1988
		EP 0257993 A	02-03-1988
		EP 0730030 A	04-09-1996
		ES 2095202 T	16-02-1997

Form PCT/ISA210 (patent family cross) (July 1999)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/26062

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5605011 A		IL 83348 A	08-12-1995
		JP 2759135 B	28-05-1998
		JP 63071184 A	31-03-1988
		NZ 221233 A	28-05-1990
		RU 2099422 C	20-12-1997
		SU 1820914 A	07-06-1993
		ZA 8706314 A	26-04-1989